DESCRIPTION OF ASEXUAL REPRODUCTION IN A TEXAS SUCCULENT *SEDUM WRIGHTII* (CRASSULACEAE)

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ABSTRACT

Sedum wrightii A. Gray, a west Texas native succulent, is one of a growing number of species known to reproduce by vegetative means. Detached leaves of *S. wrightii* produce plantlets on the basal portion of the adult leaf in association with callus tissue from what appears to be thinned-walled parenchyma and epidermal cells of the petiole. Shoot primordia appear first, emerging over lateral regions of the callus tissue by about day 5, with the roots emerging several days later.

RESUMEN

Sedum wrightii A. Gray, planta suculenta nativa de Texas, pertenece al creciente grupo de especies conocidas que se reproducen vegetativamente. Las hojas arrancadas de *S. wrightii* producen plántulas en la porción basal de la hoja adulta, en asociación con callo de lo que parece ser parénquima de paredes finas y células epidérmicas del pecíolo. Primero aparecen los primordios del vástago, emergiendo de las regiones laterales del callo sobre el quinto día, y las raíces emergen varios días más tarde.

INTRODUCTION

Survival of seeds and seedlings in shallow soils of rock outcrops (Sharitz & McCormick 1973) and on the desert soil surface is poor (Jordan & Nobel 1981), resulting in low sexual reproductive success and limiting the establishment of many desert perennials to certain favorable years. The development of young plantlets from vegetative buds borne on a parent plant, or on detached leaves occurs in several groups of plants including *Sedum*, *Bryophyllum* and *Crassula*, either normally or upon wounding (Stoudt 1934, 1938; Yarbrough 1934, 1936; McVeigh 1938). The propagative capacity of species of these genera have interested botanists, and its advantages have been exploited by horticulturists.

The development of plantlets from leaves is an efficient mechanism of vegetative reproduction in many plant families. These vegetative plantlets may be more successful than seedlings because of several factors, including greater water and carbon reserves (Holthe & Szarek 1985). In an examination of *Sedum wrightii* A. Gray, Clausen (1975) noted that after 11 weeks, 82% of detached leaves developed plantlets, each with a small rosette of leaves and well-developed roots. A later study (Gravatt 2003) showed that the additional source of water and

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carbohydrates reserves, over an extended period provided by the detached leaf, was an added benefit for the developing propagule. By day 120 of the experiment, detached leaves remained physiologically active, whereas the propagules maintained constant growth. For succulents such as *S. wrightii*, drought survival of propagules may be determined by the amount of water-storing tissue in the detached leaf.

Gravatt (2003) found that detached leaves of *S. wrightii* readily developed propagules at the base of the leaves. Each propagule develops 2 to 3 roots and a small rosette of 3 to 5 leaves. By the end of the experiment nearly 80% (98% of surviving leaves) of the detached leaves had developed propagules. The morphological events associated with asexual reproduction in *S. wrightii* have not been previously characterized. The purpose of this investigation is to describe the development of young propagules from basal callus tissue of detached, adult leaves in this species using scanning electron microscopy.

MATERIALS AND METHODS

Sedum wrightii is a leaf-succulent perennial occurring in the semi-deserts of the southwestern USA and Mexico. Plants were collected on the limestone cliffs overlooking the Devils Arm of the Amistad Reservoir, Val Verde County, Texas at an elevation of 360 m in August 2001. Plants were potted in soil sand: perlite: topsoil (3 : 3 : 3) using plastic pots (10×10 cm) and placed in a plant growth chamber. Environmental conditions were as follows: photosynthetic photon flux density (PPFD) of 900 μmol m^{-2} s^{-1}, 25($\pm$1) °C air temperature, and 1.9($\pm$0.2) kPa vapor pressure deficit (vpd) during the day, and 20($\pm$1) °C air temperature and 1.5($\pm$0.2) kPa vpd at night. Environmental conditions were measured using an ADC (Hertfordshire, England) LCA4 CO_2 and H_2O analyzer. After transplanting, plants were kept well-watered and fertilized biweekly with 0.5-strength Peters Professional fertilizer (W.R. Grace and Co., Fogelsville, PA, USA) with trace elements, 15% each of total N, K_2PO_5, and K_2O.

Plants were allowed to grow until mature, as indicated by the presence of floral buds. A voucher specimen (*Gravatt s.n.*) was deposited in the Stephen F. Austin State University Herbarium (ASTC). Leaves were detached from whole plants ("parent leaves") and were placed on moist vermiculite in plastic pots (10×15 cm) and sampled on days 0, 1, 2, 5, 8, 10, and 14. The vermiculite was kept moist in the growth chamber for the duration of the experiment. Environmental conditions inside the chamber were the same as those described for the whole plants.

On each of the sampling days the basal third of *S. wrightii* leaves were excised using a razor blade, fixed in a 1:1 mixture of glutaraldehyde (5%) and 0.1M potassium phosphate buffer and refrigerated overnight. The specimens were rinsed in deionized water and dehydrated in a graded ethanol series to 100%

ethanol (Mims 1981). The leaf pieces were then critical point dried with carbon dioxide as the transitional fluid, mounted on aluminum stubs, and sputter coated with gold or gold-palladium. Basal ends of leaves were examined using a Hitachi S-405A scanning electron microscope operating at 15 KeV.

RESULTS

The leaves of *Sedum wrightii* are spirally arranged, elliptical or oblong and glabrous. Adult leaves are 8 to 12 mm long, 3.5 to 4.5 mm wide, and 1.5 to 2.5 mm thick. Petioles are much reduced, such that they give the appearance of the leaf being nearly sessile. Preliminary findings determined that leaves of *S. wrightii* contain a single leaf trace. The petioles are very brittle, so that the leaves fall from the stem with only slight pressure and take root from the base of the leaf, forming a new plant (Fig. 1).

Scanning electron microscopic examination of the basal portion of parent leaves revealed the sequence of events associated with propagule formation. After detachment from the stem, vascular tissue and the surrounding parenchyma, as well as the epidermis of the petiole can be seen (Fig. 2). Detached leaves lying on moist vermiculite showed signs of wound tissue formation after 24 hours (Fig. 3).

Within 48 to 60 hours, a cicatrate (scar left by a wound or by abscission) meristem forms at the base of the leaf under the region of the leaf trace. The cells of the wounded surface soon collapse, and the cells underneath these divide in a plane parallel to the surface. A small protuberance, visible under a binocular microscope, appears near the region of the petiole of the parent leaf by the second or third day. This region is identified as the zone of shoot emergence in Figure 3.

When detached leaves are maintained on moist vermiculite, young shoots typically emerge from the callus tissue after five to seven days (Fig. 4). The earliest observed emergence of shoots was three days, with the latest recorded emergence at ten days. The shoot primordium arises on the lateral surface of the callus tissue. The shoot apparently originates from peripheral tissue of the petiole, at the base of the leaf, not previously covered by callus. A single shoot arises by day 5, with two primordial leaves easily identifiable (Fig. 4).

The origination of root primordia within the callus tissue follows emergence of leaf primordia and usually occurs by the eighth day (Figs. 5 & 6). Thus, the development of growing points is as follows: callus tissue forms; the shoot appears before the root; and the roots appear as single root or in pairs.

Several root primordia may be organized within a given callus tissue (Fig 6), so that by day 14 the parent leaf may have three to four adventitious roots. The development of root hairs can be seen on leaves kept on moist vermiculite by about day 14 (Fig. 7).

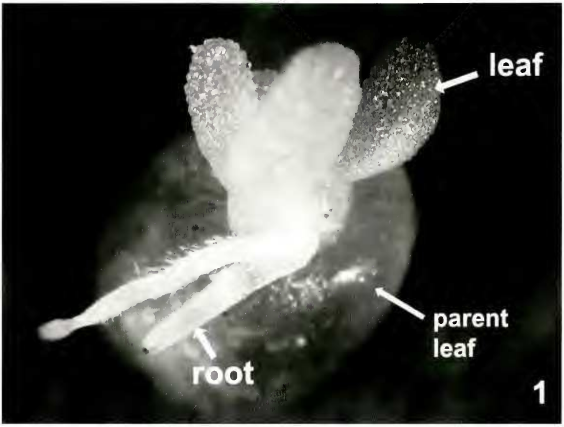


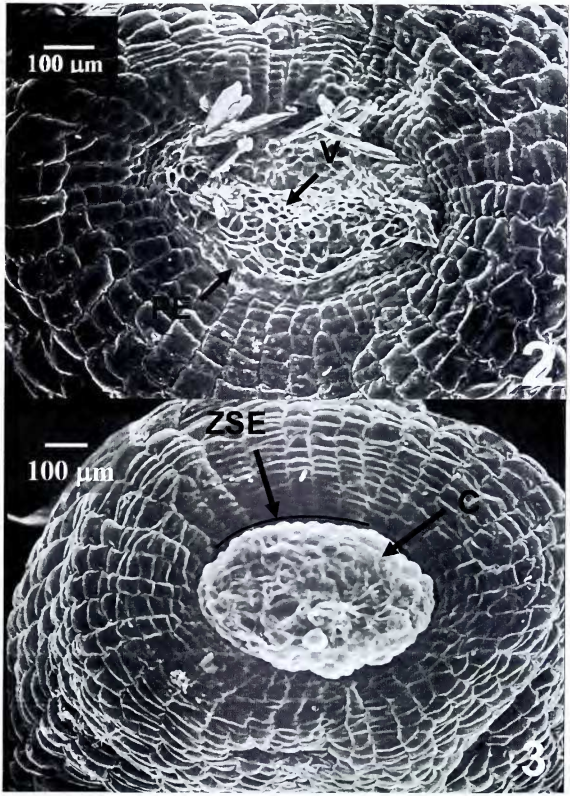
Fig. 1. Stereomicroscope image of *Sedum wrightii* leaf base with 14-day-old propagule (~30×).

DISCUSSION

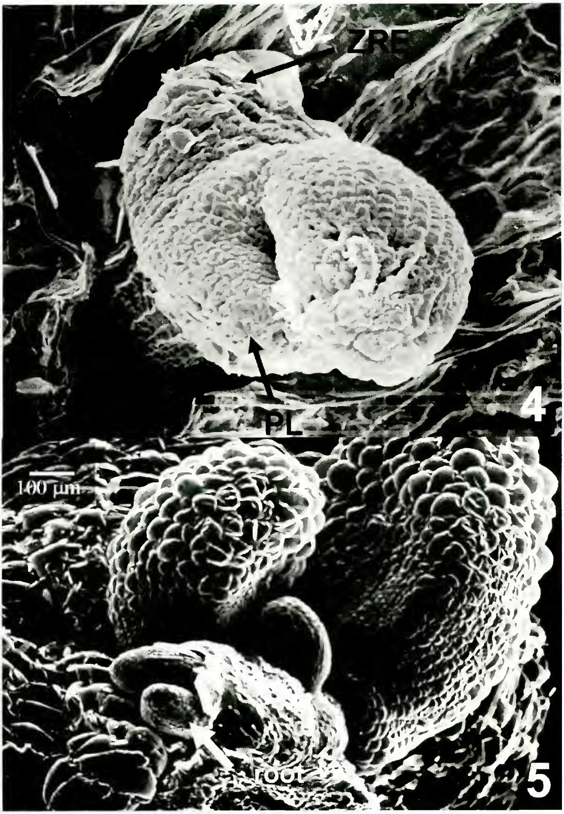
In an earlier study, Gravatt (2003) found that after 11 weeks, 82% of detached leaves developed plantlets, each with a small rosette of leaves and well-developed roots. Furthermore, detached leaves survived for 120 d with a high rate of success for propagule formation, 89% of leaves from the start of the experiment (Gravatt 2003).

Scanning electron microscopic examination of the basal portion of parent leaves revealed a sequence of events associated with propagule formation comparable to that reported in *Crassula multicava* (McVeigh 1938), *Sedum stahlii* (Yarbrough 1936), and *Graptopetalum* (Green & Brooks 1978). The basipetal meristematic activity appears to link the propagule with the vascular system, as found in previous studies (McVeigh 1938; Yarbrough 1936; Green & Brooks 1978). Thus, the propagule is linked with the parent leaf for food and water reserves (see Fig. 4), allowing survival of the young plant (Gravatt 2003).

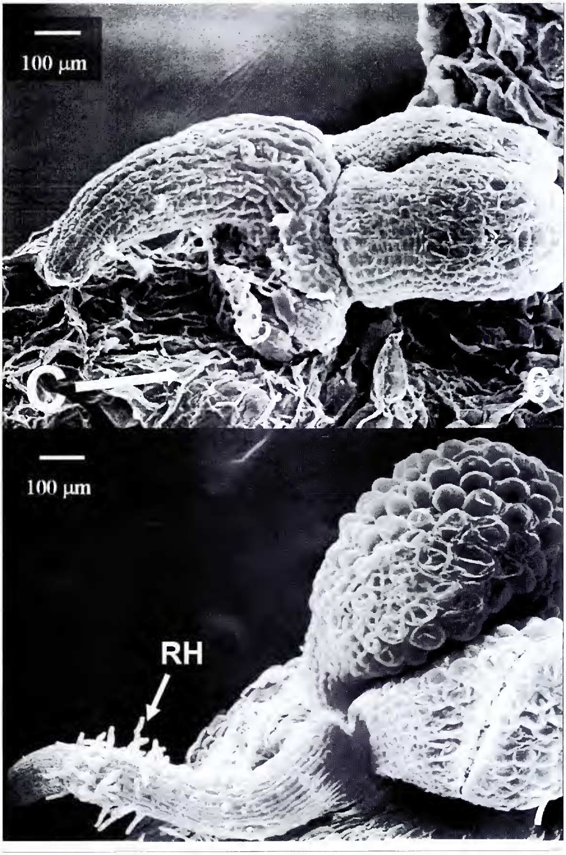
The origination of root primordia within the callus tissue follows emergence of leaf primordia and usually occurs by the eighth day (Figs. 5 & 6). These findings are in contrast to those found by Yarbrough (1936), who observed a different order of development for roots and shoots. In his study, detached leaves



Figs. 2–3. Scanning electron micrographs of *Sedum wrightii* propagules on designated sampling days. **Fig. 2.** Leaf base taken 1 day after leaf removal showing petiole epidermis and vascular tissue, **Fig. 3.** Callus tissue formed over vascular bundle on leaf base taken on day 2. (PE = petiole epidermis; V = vascular tissue; C = callus tissue; ZSE = zone of shoot emergence).



Figs. 4–5. Scanning electron micrographs of *Sedum wrightii* propagules on designated sampling days. **Fig. 4.** Emerging shoot taken on day 5 showing leaves and area of root emergence, **Fig. 5.** Emerging roots and leaves taken on day 8 with remains of callus tissue. (PL = primordial leaf; ZRE = zone of root emergence).



Figs. 6–7. Scanning electron micrographs of *Sedum wrightii* propagules on designated sampling days. **Fig. 6.** Leaf pair and elongating root taken on day 10. **Fig. 7.** Root hair formation, taken on day 14. (C = callus tissue; RH = root hairs).

of *S. stahlii*, left on a dry surface, gave rise to roots first and then shoots. Yarbrough (1936) further observed that in a moist chamber, as used in this study, shoots were not formed and roots only appeared occasionally.

Yarbrough (1936) concluded that production of roots and shoots from leaves of *Sedum stahlii*, which at the time of detachment clearly possess no residual meristem, clearly is an example of regeneration through the formation of adventitious structures. He further stated that the formation of callus tissue by the thinned-walled parenchyma cells of the short petiole involves differentiation. In this study, it appears that this is the case for *Sedum wrightii* as well, and compares well with the findings of Yarbrough (1936). The continued mitotic activity of the cells surrounding the callus makes possible the development of two fundamental growing points, the root and shoot primordia, and the subsequent development of a new plant from the detached leaf.

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